An in vitro study of manure composition on the biochemical origins, composition, and accumulation of odorous compounds in cattle feedlots^{1,2}

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ABSTRACT: Very little is known about the biochemical origin of cattle feedlot odors and the environmental factors controlling their production. The tie between diet and manure composition is well established, but the effect of different manure compositions on odorous chemical production is unknown. This study describes the effect of starch, casein, and cellulose substrate additions to slurries of fresh (< 24 h) and aged cattle manure (> 1 d) on the anaerobic production of fermentation products and the consumption of substrates relative to no addition treatments. Aged cattle manure accumulated more VFA (245 to 290 m*M*) than the fresh manure (91 to 181 mM) irrespective of substrate additions (P < 0.001). In fresh manures, VFA concentrations were increased (P < 0.01) over no addition treatments when carbohydrate (starch or cellulose) was added, whereas starch and protein treatments to aged manure increased VFA content relative to no addition treatments (P < 0.001). Branched-chain VFA and aromatic compounds accumulated only in the aged manure (no addi-

tion and protein treatments), indicating that some protein fermentation occurred in those treatments. Based upon substrate loss, starch fermentation was the dominant process in both manures and all treatments with losses exceeding 18.6 g/L. Protein fermentation occurred only in the aged manure, specifically the no addition and protein treatments, when starch was no longer available. The production of odorous compounds from manure was controlled by substrate availability and pH, with pH related to lactate accumulation. We believe that calcareous soil and lactate-consuming microorganisms in the aged manure slurries minimized slurry acidification and resulted in greater accumulations of odorous products. Substrate additions had little effect on the overall accumulation of odor compounds in manure but had profound effects on odor compound composition. We propose that modifying cattle diets to limit starch and protein excretion would profoundly affect the production and accumulation of odor compounds in feedlots.

Key Words: Bacteria, Cattle, Manures, Odors

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Introduction

Microorganisms play a central, yet poorly characterized, role in the production of agricultural odors in cattle feedlots. Bacteria carry out an incomplete anaerobic fermentation of substrates in livestock manures (Mackie et al., 1998), producing a complex chemical mixture of VFA, alcohols, aromatic compounds, amides (including NH₃), and sulfides (O'Neill and Phillips,

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1992; Hartung and Phillips, 1994). Volatilized fatty acids and aromatic compounds most closely correlate to odor (Zahn et al., 1997, 2001; Powers et al., 1999); therefore, limiting the source concentrations of these compounds in manures would help to control odor emissions. Manure microorganisms have access to a wide variety of potential substrates, including starch, proteins, lipids, and nonstarch carbohydrates, to produce VFA and aromatic compounds (Mackie et al., 1998; Zhu et al., 1999). Recently, evidence for a preferred substrate for fermentation (starch) has been suggested from the mix of available substrates in cattle manure (Miller and Varel, 2001). In that study, protein fermentation was very limited and observed only at the end of the slurry fermentation in older manures when starch was depleted. Fresh manures never became starch-depleted, most likely because lactate accumulation decreased the pH enough to inhibit manure fermentation. We hypothesize that 1) the addition of substrates to fresh cattle manure will not affect the accumulation and composition of fermentation products,

¹Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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because fermentation of starch to lactate is preferred and self-limiting; and 2) addition of starch to aged manure will compensate for low starch levels and inhibit protein fermentation, whereas the addition of protein would only stimulate protein fermentation after starch was depleted. The objective of this study was to evaluate the effect of manures with differing substrate compositions on the production of odorous fermentation products in fresh and aged manure slurries.

Materials and Methods

Manure Slurry Incubations and Analyses

Aged manure and fresh (i.e., had not formed a crust) feces samples were collected from six adjacent feedlot pens at an open-air cattle feedlot during September 2000 as previously described (Miller and Varel, 2001). Aged manure samples consisted of a mixture of loose soil and dried, unconsolidated manure. For manure slurries, 1 kg of manure (fresh or aged) was blended in a Waring blender (New Hartford, CT) with 700 mL of cattle urine, 2.8 g urea, and 3.3 L of 12.12 mM H₂KPO₄ (pH 7.5). The urea and phosphate buffer were added to the urine to replenish urea hydrolyzed during storage and to neutralize the acid used for preservation. For each of the manures, the slurry was equally divided into four 1-L samples and poured into a blender. No additions were made to the first blender. Casein (10.3 g) was added to the second blender. Starch (25.8 g) was added to the third blender, and microcrystalline cellulose (51.6 g) was added to the fourth blender. The quantities of the additions were based upon a doubling of the endogenous concentration in fresh manure. Each sample was blended, and approximately 180 mL of manure slurry was then added to five 250 mL flasks (five replicate flasks per manure/treatment). Flasks were gassed with N₂, stoppered to limit volatilization losses and ensure anaerobic incubations conditions, and then incubated at room temperature (20 to 23°C). Excess fermentation gas was vented though a needle into a water-filled test tube. At periods ranging from daily to weekly, slurry samples were collected. Manure slurry pH was analyzed immediately using a combination pH electrode, whereas the remaining manure parameters (substrates and fermentation products) were determined from frozen samples analyzed after the end of incubation. Details of the analyses have been previously described (Miller and Varel, 2001). Briefly, manure substrates (nonammonia nitrogen (NAN), starch, nonstarch carbohydrate) were analyzed in homogenized samples. Samples for NAN content, analogous to fermentable nitrogen (protein and nucleic acids), were made alkali and dried overnight at 100°C to remove free NH₃ before analysis using a LECO CN-2000 carbon/ nitrogen analyzer (LECO, St. Joseph, MI). Starch was measured using membrane-immobilized enzyme system (YSI Model 27, Yellow Springs Instrument Co., Yellow Springs, OH) as free glucose after overnight

digestion with amyloglucosidase. Total carbohydrate was determined colorimetrically using the phenol-sulfuric acid reaction (Daniels et al., 1994). Fermentation products (L-lactate, ethanol, propanol, isobutanol, butanol, pentanol, hexanol, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, heptanoate, caprylate, phenol, ρ -cresol, 4-ethyl phenol, indole, skatole, benzoate, phenylacetate, and phenylpropionate) were quantified in the liquid phase of the slurries using the YSI analyzer for L-lactate and a Hewlett Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with flame ionization and mass selective detectors for all other products. Conditions used for separating the fermentation products have been previously described (Miller and Varel, 2001).

Statistical Analyses

Data were analyzed as a split plot in time. The unit of observation was the flask (n = 40), and there were no missing values. The model included effects of manure type, incubation time, treatment, flask (manure type \times treatment), manure type \times incubation time, manure type \times treatment, incubation time \times treatment, and manure type \times treatment \times incubation time. Manure type, treatment, and manure type × treatment were tested against the mean square of flask (manure type × treatment). Differences between least-squares means were tested with a protected t-test. Data for percent molar composition of selected fermentation products were also analyzed using this model, with the exception that the initial (d 0) percent composition for all treatments were pooled and handled as an additional treatment in the model. This allowed subsequent incubation times to be tested against the initial composition. Statistical analyses were conducted with the GLM procedure of SAS v. 7.0 (SAS Inst. Inc., Cary, NC).

Results and Discussion

Production of Odor Compounds and Fermentation Products in Manure Slurries

Addition of substrates had a substantial impact on the accumulation of total alcohol, VFA, and aromatic ring compounds, depending upon the substrate and manure (Figure 1). The pattern of alcohol accumulation was similar, irrespective of treatment or manure, with most accumulation occurring during the first 10 d (Figure 1A, B). Alcohol concentrations decreased (P < 0.001) after d 10 in all treatments and manures with the exception of aged-starch and aged-cellulose, which tended to increase ($P \le 0.06$). The rate of alcohol loss after d 10 tended to vary between manures ($P \le 0.09$) with fresh manures (all treatments) consuming alcohols at a lower rate (range: 0.55 to 0.34 mM/d) than aged-no addition and aged-protein treatments (1.29 and 0.78 mM/d, respectively). Total alcohol concentrations at the end of

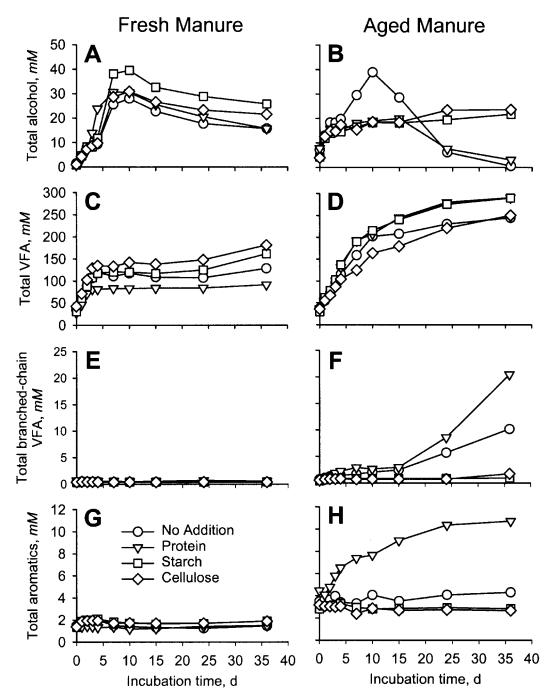


Figure 1. Concentrations of alcohols, VFA, branched-chain VFA, and aromatic compounds during incubation of fresh and aged cattle manure slurries. The SE of the least squares means (n = 5) for total alcohols is 1.3, for total VFA is 7.1, for total branched-chain VFA is 0.64, and for total aromatics is 0.21. All ANOVA for manure type \times treatment \times incubation time differed at P < 0.001. Data for no addition treatments in Figures 1 to 4 were previously reported (Miller and Varel, 2001) and are presented for comparison.

incubation were highest when carbohydrates were added to the manures compared to no addition and protein treatments ($P \le 0.001$).

Volatile fatty acids accumulated (P < 0.001) during the first 5 d in both manures (Figure 1C, D), but in fresh manure, VFA accumulation slowed and increased only slightly after d 4 in the starch and cellulose treatments (P < 0.001). In the aged manure slurries, VFA

accumulation continued throughout the incubation, but slowed by the end of the incubation period. Peak accumulations of VFA varied by treatment and manure, with aged manure producing higher concentrations of VFA than the fresh manure (P < 0.001). Carbohydrate (starch or cellulose) additions to the fresh manure produced higher ($P \le 0.001$) VFA concentrations than freshno addition controls, whereas aged-starch and aged-

Table 1. Initial and final molar percent composition of important fermentation products

Fermentation product	Manure type	$\begin{tabular}{ll} Initial molar percent \\ \hline composition^b \\ \hline \\ All treatments \\ \hline \end{tabular}$	Final molar percent composition for each treatment after 36 d of incubation				
			No addition	Protein	Starch	Cellulose	SE
Ethanol	Fresh	88.9°	93.7^{cd}	$93.3^{ m cd}$	93.7^{cd}	$97.0^{ m d}$	1.5
	Aged	98.1^{c}	$40.4^{ m d}$	55.3^{e}	$97.6^{ m cf}$	$92.3^{ m f}$	1.5
Acetate	Fresh	$56.0^{ m c}$	$28.7^{ m d}$	$48.0^{\rm e}$	$5.4^{ m f}$	$4.4^{ m f}$	1.1
	Aged	63.2^{c}	39.9^{d}	$35.4^{ m d}$	$27.5^{\rm e}$	$22.1^{\rm f}$	1.1
Propionate	Fresh	$25.1^{\rm c}$	18.1^{d}	19.6^{e}	$15.2^{ m f}$	$16.5^{\rm g}$	0.2
	Aged	$20.8^{\rm c}$	$12.0^{ m d}$	11.1^{d}	9.0^{e}	$9.8^{\rm e}$	0.2
Butyrate	Fresh	$16.2^{ m c}$	$52.6^{ m d}$	$31.3^{\rm e}$	78.7^{f}	78.6^{f}	1.2
	Aged	12.5°	$39.3^{ m d}$	$40.4^{ m d}$	52.6^{e}	$62.3^{ m f}$	1.2
Branched-chain VFA ^a	Fresh	$1.3^{\rm c}$	$0.3^{ m d}$	$0.6^{ m cd}$	$0.3^{ m d}$	$0.2^{ m d}$	0.2
	Aged	$1.5^{ m c}$	$4.2^{ m d}$	$6.8^{\rm e}$	$0.3^{ m f}$	$0.7^{ m f}$	0.2

^aSum of isobutyrate, isovalerate, and isocaproate.

 c,d,e,f,g Least-squares means of initial (n = 20) and final (n = 5) molar percent composition within a row without a common superscript letter differ ($P \le 0.001$).

protein treatments accumulated more VFA than the aged-no addition treatment (P < 0.001).

Branched-chain VFA, which included isobutyrate, isovalerate, and isohexanoate, are associated with manure odors and have very low odor thresholds (Zahn et al., 2001). The accumulation of these compounds varied between manures and treatments (Figure 1 E, F). None of the treatments in the fresh manure accumulated any branched-chain VFA (P > 0.89), whereas two of the treatments in the aged manure (aged-no addition and aged-protein) accumulated substantial quantities of branched-chain VFA over initial concentrations (P < 0.001).

The concentrations of aromatic compounds (phenols, indoles, and benzoates) differed initially and showed a different pattern of production between the two manures (Figure 1G, H). Aged manure contained a higher initial concentration of aromatics than fresh manure (P < 0.001). Aromatic compound accumulation $(P \le 0.01)$ occurred in only two treatments of the aged manure (aged-no addition and aged-protein treatments), whereas there was no accumulation in any of the treatments where fresh manure was tested. In the aged-protein treatment, phenolic compounds (phenol, cresol, and 4-ethyl phenol) and benzoate compounds (benzoate, phenyl acetate, and phenyl propionate) increased (P < 0.001) by 433% and 163%, respectively.

Although the concentration of odorous chemical compounds generally increased during the incubation, the molar percent composition of individual compounds changed drastically (Table 1). Ethanol was the greatest contributor to the total pool of alcohols, making up more than 85%, initially, and at the end of the incubation (Table 1). The percentage of ethanol changed very little from initial to final composition with two notable exceptions, aged-no addition and aged-protein treatments, where the percentage of ethanol was lower (P < 0.001).

In those treatments, there was nearly complete alcohol consumption, and based upon the final composition of alcohols, we conclude that ethanol was preferentially consumed. The role of alcohols in feedlot odors is unclear. Whereas alcohol is a major fermentation product, it does not affect odor intensity, rather it modifies odor quality (Yasuhara, 1980). Another unexplored role for alcohols may be to act as a solvent for odor compounds, which may increase or decrease their volatility. More research needs to be conducted on the relationships between high alcohol concentrations and odor compound production and accumulation in manures.

Although the molar concentrations of total VFA increased throughout the course of the study (P < 0.001). the molar percent composition of individual VFA changed markedly depending upon incubation time, substrates added, and manure age (Table 1). The VFA pool was largely dominated by acetate, propionate, and butyrate, which together made up from 86.9 to 99.5% of the total VFA pool during the study. Although initial VFA composition differed (P < 0.001) between fresh and aged manures (except for total branched-chain VFA), there was a general trend for the VFA pool in both manures to initially have a higher molar percentage of acetate compared to propionate and butyrate. By the end of incubation, the percentage of butyrate increased (P < 0.001) and was the largest contributor to the VFA pool with the exception of the fresh-protein and agedno addition treatments. The molar percentage of acetate and propionate decreased (P < 0.001) in all treatments and manures even though their molar concentrations increased. The greatest decreases in percentage of acetate were often associated with large increases in the percentage of butyrate, particularly in the starch and cellulose treatments for both manures. The molar percentage of total branched-chain VFA was also highly dependent on manure and treatment. The percentage of

^bExpressed as the molar percent composition of the total molar pool of alcohol (for ethanol) or VFA (for acetate, propionate, butyrate, and branched-chain VFA). Manure types differed (P < 0.001) in initial molar percentage of ethanol, acetate, propionate, and butyrate (SE of the initial molar percent LS means (n = 20) are 0.7, 0.5, 0.1, 0.6, and 0.1 respectively). All ANOVA for manure type × treatment × incubation time interactions are significant (P < 0.001). Probability of a Type I error within fermentation product is 0.021.

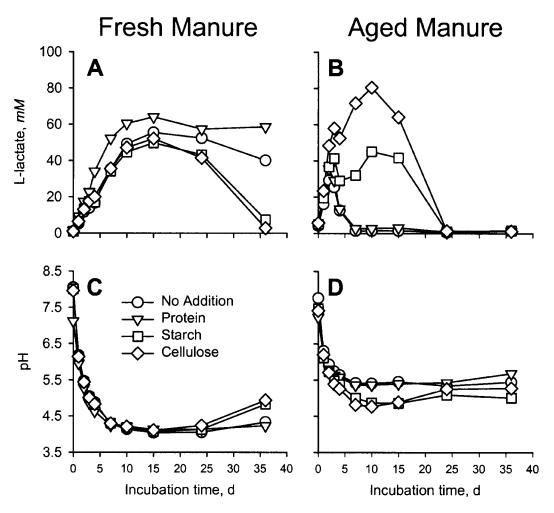


Figure 2. Concentrations of L-lactate and slurry pH during the incubation of fresh and aged cattle manure slurries. The SE of the least squares means (n = 5) for L-lactate is 1.56 and for pH is 0.025. All ANOVA for manure type \times treatment \times incubation time differed at P < 0.001.

branched-chain VFA decreased in starch and cellulose treatments for both manures (P < 0.001), whereas the percentage of branched-chain VFA increased in no addition and protein treatments in the aged manure (P < 0.001). This suggests that treatment not only affected the final molar concentration of odorous chemical compounds, but it also affected the final composition of odorous compounds. We hypothesize that the effect of altered odor compound composition caused by differing manure compositions will have a strong effect on perceived odor.

Lactate, pH, and Odor Compound Accumulation

The accumulation of acid end-products limits the further production of VFA in fresh manure slurries (Miller and Varel, 2001). Lactate accumulated and persisted in both the fresh and aged manure slurries to varying degrees (Figure 2A, B), and had a noticeable influence on slurry pH (Figure 2C, D). Lactate accumulated (P < 0.001) in the fresh manure slurries (fresh-no addition and fresh-protein treatments) and persisted throughout the incubation, driving the pH below 4.75 and inhib-

iting further production of VFA. However, acid-tolerant microorganisms in two of the fresh manure treatments (fresh-starch and fresh-cellulose), apparently converted lactate into VFA at the end of the incubation and caused slurry pH to rise above 4.75. A similar relationship between lactate accumulation, pH, and VFA accumulation was observed in the aged manure (aged-starch and aged-cellulose treatments). When lactate concentrations in these two treatments were highest, the pHs were lowest, and when lactate was consumed, the pH levels increased (P < 0.001). Although the lactate concentration was highest in the aged-cellulose treatment compared to fresh manure slurries (P < 0.001), fresh manure slurries had much lower pH values (P < 0.001). It is likely that the calcareous soil in the aged manure samples helped buffer the pH in those slurries. A direct consequence was that the pH of the aged manure slurries never fell below the pH 4.75 threshold where undissociated VFA disrupts the proton motive force of bacterial membranes and inhibits microbial activities (Switzenbaum et al., 1990). We conclude that the accumulation of lactate and subsequent pH decrease limits malodorous VFA accumulations in fresh manures regardless of the manure substrate composition. Lactate accumulation and pH did not affect VFA accumulation in aged manures in this study, although there were differences (P < 0.05) in lactate concentrations depending upon the substrate composition of the manure.

Origins of Manure Odor Compounds

Three lines of evidence (initial substrate composition, production of specific fermentation products, and changes in substrate composition during the incubation) can be used to ascertain the probable source of manure odor compounds. In an earlier study, we reported that the initial composition of the manures in the no addition treatments indicated that compared to fresh manure, the aged manure was depleted in starch and tended to have higher protein concentrations (Miller and Varel, 2001). We propose that there was some starch fermentation several days before sampling the aged manure that occurred as the freshly deposited manure dried out and mixed with the feedlot surface soil during the aging process.

A primary objective of this study was to determine whether changing the availability of substrates in cattle manures could modify manure fermentations into different metabolic pathways (protein vs starch) to produce different compositions or amounts of odorous chemicals. Production of branched-chain VFA and aromatic compounds have been linked to protein fermentation (Mackie et al., 1998). Studies of mixed culture microbial fermentations in the human colon demonstrate that protein fermentations for both energy production and cell biosynthesis typically produce a significant percentage of branched-chain VFA (7 to 17%) relative to total VFA and substantial increases in aromatic compounds (Smith and Macfarlane, 1996; 1998). No substrate additions were able to stimulate the production of branch-chained VFA or aromatic compounds in fresh manure (Figure 1E, G); thus, we conclude that protein was not the preferred substrate for odor compound formation in fresh manure. In aged manure, the accumulation of branched-chain VFA and aromatic compounds (Figure 1F, H) was observed in two treatments (no addition and protein) indicating some protein fermentation was occurring. The addition of carbohydrate (starch or cellulose) appeared to limit protein fermentation and suggests that fermentative bacteria prefer carbohydrate to protein for energy and biomass production. We conclude that manures of differing substrate content or availability will follow different metabolic pathways and produce fermentation products differing in composition and odor offensiveness.

Clear evidence for starch fermentation in all treatments and manures is presented in Figure 3. The accumulation of fermentation products including VFA, alcohols, and lactate (Figure 3A, B) was closely tied to starch consumption (r = 0.936 \pm 0.007; n = 40) compared to nonammonia nitrogen (NAN) (r = -0.049 \pm 0.087; n

= 40), a marker for fermentable nitrogen (primarily protein), or nonstarch carbohydrate ($r = -0.017 \pm 0.080$; n = 40). Starch was consumed in all treatments (P <0.001), but some starch remained (i.e., differed from 0 at $\alpha = 0.05$) at the end of incubation in all fresh manure slurries and in the aged-starch treatment, which had added starch. The pattern for starch consumption was very similar for all treatments except the aged-starch treatment (Figure 3D). Nearly twice the starch was consumed in the aged-starch treatment compared to the other treatments (P < 0.001). This treatment produced only slightly more fermentation product than the other treatments $(P \le 0.03)$ with the exception of the aged-protein, which did not differ (P = 0.16) in fermentation product content from the aged-starch treatment (Figure 3B). The imbalance may be due to the production of new bacterial biomass or bacterial noncarbohydrate storage polymers.

If protein fermentation was the primary metabolic pathway for anaerobic manure decomposition (i.e., no starch fermentation), a large fraction of the fermentable nitrogen would have been utilized for energy production resulting in decreased nonammonia nitrogen and increased free ammonia. Nonammonia nitrogen levels decreased (P < 0.05) in only two treatments of the aged manure (Figure 3E, F). Nonammonia nitrogen decreased from initial concentrations by 24.2% in the aged-no addition treatment and by 16.2% in the agedprotein treatments, which was consistent with protein fermentation playing an important, but peripheral role in manure fermentation. In all other treatments, the NAN remained unchanged (P > 0.1) from initial concentrations. The accumulation of protein fermentation products (branched-chain VFA and aromatic compounds) was limited exclusively to those treatments where nonammonia nitrogen content decreased. Production of the protein-specific products was observed in manure slurries where starch was completely consumed or when easily fermented protein was available (aged-protein treatment). Nonstarch carbohydrate was unchanged (P > 0.2) in all treatments and manures except the aged-no addition treatment, where nonstarch carbohydrate decreased (P < 0.05) during the incubation (Figure 4G, H). This data confirms that starch is preferentially consumed in cattle manures, followed by protein consumption. Furthermore, adding starch to starch depleted manures temporarily circumvents protein fermentation and the formation of highly objectionable branched-chain VFA and aromatic compounds.

Our original hypotheses tested under the conditions of this experiment were largely correct. Odor compound production in fresh cattle manure was primarily from starch fermentation and was self-regulating due to lactate build-up and low pH. Although total VFA was lower in the fresh-protein treatment compared to other treatments, it was probably related to an initially lower pH; hence, the VFA inhibition threshold was reached sooner in that particular treatment. Some of the results related

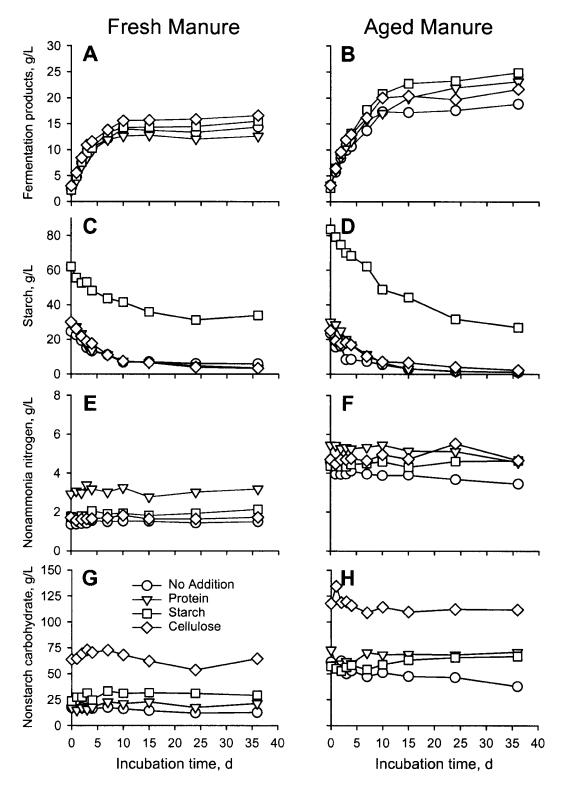


Figure 3. Relationships between fermentation product production (alcohols + VFA + L-lactate) and protein, starch, and nonstarch carbohydrate content in the manure slurries during incubation. The SE of the least squares means (n = 5) for fermentation products is 0.56, for starch is 1.43, for NAN is 0.78, and for nonstarch carbohydrate is 5.5. All ANOVA for manure type × treatment × incubation time differed at P < 0.01 with the exception of nonstarch carbohydrate (P = 0.962).

to our first hypothesis were unexpected. Carbohydrate additions to the fresh manure had an impact on the composition of fermentation products, particularly butyrate and lactate at the end of incubation. Further research is needed to determine the mechanisms responsible. Our second hypothesis was correct; addition of starch to aged manure inhibited protein fermentation and the production of branched-chain VFA and aromatic compounds. Unexpectedly, protein additions to aged manure stimulated VFA accumulation to the same extent as starch additions. It is possible that in aged manure there is a low rate of protein fermentation, and the addition of highly soluble protein (casein) stimulated that activity. Results of these experiments may be biased due to the experimental setup—high moisture slurries in a closed flask system. Additional studies are underway in our laboratory to validate the results of this study and examine the production of odorous chemicals during cattle manure fermentation in cattle feedlot soils under more realistic moisture and oxygen conditions.

Dietary Considerations and Feedlot Odors

A link between cattle diet and manure odor has been described in a few studies (Kellems et al., 1979; Watts and Tucker, 1993; Watts et al., 1994), but the mechanism responsible for manure odor production is poorly understood. Recent molecular studies indicate that Clostridium, Lactobacillus, and Bacillus microorganisms are likely responsible for manure decomposition and odor production (Ouwerkerk and Klieve, 2001; Whitehead and Cotta, 2001). It is likely that differences in the microbial community between fresh and aged manure account for part of the differences in odor compound accumulation, but much more research needs to be conducted before the relationships between microbial community structure and odor compound production are fully appreciated in the cattle feedlot environment.

The link between manure composition and odor formation is becoming clearer. In studies by Watts and Tucker (1993) and Watts et al. (1994), starch content in the fresh feces was strongly related to odor intensity of wetted feedlot pads. Our results demonstrate that microorganisms preferentially fermented starch into a variety of odor compounds, even when other substrates (protein or microcrystalline cellulose) were available. However, predicting odor potential at cattle feedlots may not be as simple as measuring starch content in the manure. Once starch is depleted, protein fermentation in aged manure is likely and may result in the production of more objectionable odor chemicals that include branch-chain VFA, phenolic compounds, and indole compounds, which have very low odor thresholds (Zahn et al., 2001). A combination of lower starch content and less available protein in the fresh manure should limit odor compound formation during both the initial manure fermentation and later fermentations when the feedlot pen becomes wet due to rainfall events (Watts and Tucker, 1993; Watts et al., 1994).

Diet composition would play a central role in feedlot odors at three distinct points. First, fresh manure contains a variety of odor compounds produced in the animal during digestion and includes products of carbohydrate (starch and cellulose) and protein fermentation. Second, initial fermentation in the fresh manure would occur during the next few days as manure dries out and mixes with underlying soil. Based upon our results, we believe that this would be a minor source for odor, as fresh manure would only cover a small fraction of the feedlot surface, usually near the feed bunk. Starch fermentation would dominate in fresh manure and produce a mixture of lactate, acetate, propionate, and butyrate. Low pH in the fermented fresh manure would enhance VFA volatility from this source. Third, rainfall events would trigger a secondary fermentation. The types of odorous compounds and their accumulations would vary based upon substrate composition (i.e., whether starch was depleted and protein was available), but odorous chemical compound accumulation could occur over the whole feedlot surface and represent a much larger source on an area basis than accumulations of fresh manure near the feed bunk. Better odor management at cattle feedlots can be accomplished if we develop a better understanding of the relationships between cattle diet, manure composition, and manure fermentation by soil and fecal microorganisms.

Implications

Substrate additions to fresh and aged feedlot manures confirmed that manure odor compounds originate primarily from starch fermentation rather than protein or other carbohydrate (i.e., cellulose) fermentation. However, protein fermentation does take on a larger role in aged manures and can be a source of very obnoxious odor compounds. Addition of substrates to both fresh and aged manures can also impact the final composition of odor compounds with excess carbohydrate stimulating the accumulation of butyrate. Lactate accumulation in fresh manures vs aged manures was also a significant factor in the overall accumulation of total VFA. These results suggest that the ties between cattle diet, manure composition, and microbial odor production are important, and investigations of these relationships are paramount for better odor control at cattle feedlots.

Literature Cited

Daniels, L., R. S. Hanson, and J. A. Phillips. 1994. Chemical Analysis.
In: P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg
(ed.) Methods of General and Molecular Bacteriology. pp 512–554. American Society for Microbiology, Washington, DC.

Hartung, J., and V. R. Phillips. 1994. Control of gaseous emissions from livestock buildings and manure stores. J. Agric. Eng. Res. 57:173–189.

Kellems, R. O., J. R. Miner, and D. C. Church. 1979. Effect of ration, waste composition, and length of storage on the volatilization of ammonia, hydrogen sulfide, and odors from cattle waste. J. Anim. Sci. 48:436–445.

Mackie, R. I., P. G. Stroot, and V. H. Varel. 1998. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. 76:1331–1342.

Miller, D. N., and V. H. Varel. 2001. In vitro study of the biochemical origin and production limits of odorous compounds in cattle feedlots. J. Anim. Sci. 79:2949–2956.

- O'Neill, D. H., and V. R. Phillips. 1992. A review of the control of odour nuisance from livestock buildings: Part 3. Properties of the odorous substances which have been identified in livestock wastes or in the air around them. J. Agric. Eng. Res. 53:23–50.
- Ouwerkerk, D., and A. V. Klieve. 2001. Bacterial diversity within feedlot manure. Anaerobe 7:59–66.
- Powers, W. J., H. H. Van Horn, A. C. Wilkie, C. J. Wilcox, and R. A. Nordstedt. 1999. Effects of anaerobic digestion and additives to effluent or cattle feed on odor and odorant concentrations. J. Anim. Sci. 77:1412–1421.
- Smith, E. A., and G. T. Macfarlane. 1996. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effect of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. J. Appl. Bacteriol. 81:288–302.
- Smith, E. A., and G. T. Macfarlane. 1998. Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiol. Ecol. 25:355–368.
- Switzenbaum, M. S., E. Giraldo-Gomez, and R. F. Hickey. 1990. Monitoring of the anaerobic methane fermentation process. Enzyme Microb. Technol. 12:722–730.

- Watts, P. J., M. Jones, S. C. Lott, R. W. Tucker, and R. J. Smith. 1994. Feedlot odor emissions following heavy rainfall. Trans. ASAE (Am. Soc. Agric. Eng.) 37:629–636.
- Watts, P. J., and R. W. Tucker. 1993. The effect of ration on waste management and odour control in feedlots. In: D. J. Farrell (ed.) Recent Advances in Animal Nutrition in Australia. pp 117–129. University of Armidale, NSW, Australia.
- Whitehead, T. R., and M. A. Cotta. 2001. Characterization and comparison of microbial populations in swine feces and manure storage pits by 16S rDNA gene sequence analyses. Anaerobe 7:67–70.
- Yasuhara, A. 1980. Relation between odor and odorous components in solid swine manure. Chemosphere 9:587–592.
- Zahn, J. A., A. A. Dispirito, Y. S. Do, B. E. Brooks, E. E. Cooper, and J. L. Hatfield. 2001. Correlation of human olfactory responses to airborne concentration of malodorous volatile organic compounds emitted from swine effluent. J. Environ. Qual. 30:624-634.
- Zahn, J. A., J. L. Hatfield, Y. S. Do, A. A. DiSpirito, D. A. Laird, and R. L. Pfeiffer. 1997. Characterization of volatile organic emissions and wastes from a swine production facility. J. Environ. Qual. 26:1687–1696.
- Zhu, J., G. L. Riskowski, and M. Torremorell. 1999. Volatile fatty acids as odor indicators in swine manure—a critical review. Trans. ASAE (Am. Soc. Agric. Eng.) 42:175–182.